Supercooling and cold-hardiness in eggs of western and northern corn rootworms

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Abstract

Oviposition by northern corn rootworms, *Diabrotica barberi* Smith and Lawrence, and western corn rootworms, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), key pests of corn in the Great Plains of the USA, occurs in the soil during late summer. Overwintering eggs are exposed to variable soil moisture and temperatures below −5 °C. The winter mortality of eggs in the soil is a primary factor that determines the potential for larval injury to corn the following spring. Our studies aimed to determine the comparative supercooling capacities of northern and western corn rootworm eggs and to assess egg mortality following brief exposure to extreme low temperature, ranging from −12.0 to −21.5 °C, under three moisture regimes. Eggs of northern corn rootworm were supercooled to a temperature as low as −27 °C, and survived supercooling to a greater extent than did western corn rootworm eggs. Moisture treatment prior to supercooling had little effect on northern corn rootworm eggs. Western corn rootworm eggs were more resistant than northern corn rootworm eggs to the effects of desiccation followed by supercooling. The survival of northern corn rootworm eggs was better than western corn rootworms under dry conditions, followed by exposure to temperatures of −12.0 and −17.5 °C, but was very low at −21.5 °C, regardless of the moisture regime. The results suggest that moisture and temperature may interact in the soil environment to determine the overwintering survival of corn rootworms. It is evident from these studies that both rootworm species experience mortality at temperatures well above the supercooling points of the eggs, but that differences exist in the effects of substrate moisture treatments on the cold-hardiness of eggs from the two species.

Introduction

The corn rootworm complex (Coleoptera: Chrysomelidae) in the northern Great Plains of the USA consists of two species, northern corn rootworms, *Diabrotica barberi* Smith and Lawrence, and western corn rootworms, *D. virgifera virgifera* LeConte. Northern corn rootworms are indigenous to the prairie grasses of the northern Great Plains (Krysan, 1993). Western corn rootworms are thought to be of tropical origin (Krysan, 1982) and probably pre-adapted to cold temperatures by a diapause capability for survival under seasonal desiccation in the tropics. Both species overwinter as eggs in the soil, where subfreezing temperatures may cause mortality (Chiang, 1965; Calkins & Kirk, 1969; Chiang, 1974). Consequently, their severity of infestation in the following summer depends greatly on the overwintering survival of the egg stage.

Relatively little is known about the cold-hardening and supercooling capacity of corn rootworm eggs. Earlier studies often focused on determining temperature limits for development, and on defining optimal chilling conditions for the storage of diapausing eggs during laboratory rearing (Ellsbury et al., 1998). Patel & Apple (1967) found that egg hatching was reduced at −2 °C and prevented when eggs were held at −10 or −23 °C for 6 or more weeks. Gustin (1981) established a winter soil temperature of −7.5 °C at depths of 7.5–15 cm as a lower temperature limit below which a significant mortality of eggs was observed.

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In spite of the rigors of the winter soil environment, a sufficient proportion of corn rootworm eggs survive winter conditions in the corn-growing areas of North America to produce significant economic infestations. Predictive models have been determined for egg hatch of the western (Woodson & Gustin, 1993) and northern (Woodson & Ellsbury, 1994) corn rootworm in response to the intensity and duration of exposure to sub-zero temperatures. A linear multiple regression model of egg hatch for western corn rootworm (Woodson & Gustin, 1993) showed that as the temperature decreased, mortality increased progressively with duration of exposure. Woodson & Ellsbury (1994) characterized northern corn rootworm egg hatch using a curvilinear model. Predictions from this model agree within ± 2 SE with survival data from northern corn rootworm eggs (Gustin, 1983) acclimated in the field prior to laboratory exposure to a temperature as low as –10 °C for up to 6 weeks.

Our studies aimed to determine the supercooling points of western and northern corn rootworm eggs, and to determine effects on egg survival of exposing eggs to varying soil substrate moisture levels followed by brief exposure to temperatures below the –10 °C threshold of Gustin (1983).

Materials and methods

Egg sources

Western corn rootworm eggs were produced from field-collected adults in the rearing facility of the Northern Grain Insects Research Laboratory, USDA, ARS, Brookings, SD. Northern corn rootworm eggs were produced from field-collected adults by French Agricultural Research Inc., Lamberton, MN. Eggs were held for 14 days in 80-mesh clay loam soil at 25 °C for prediapause embryonic development and stored at 8 °C for ca. 5 months prior to experimental use.

Moisture treatments

To simulate variable soil moisture environments, groups of 200 eggs were placed on Whatman no. 3 qualitative filter paper discs (7 cm diameter) and held in plastic Petri dishes (9 cm diameter). Three moisture levels were attained by applying distilled water to the filter paper as follows: dry, no water; moist, 1.5 ml; and wet, 3.0 ml. Eggs were transferred to the filter paper surface using a 000 paint brush and the Petri dishes were sealed with Parafilm to their maintain moisture levels.

The mean ± SE moisture content (% wet mass) of the eggs was determined for both species. Means were calculated from five replicates of five eggs each. Means were compared within the northern and western corn rootworm populations by ANOVA, and significant differences were determined by Bonferroni test.

Determination of the supercooling point

A filter paper carrying eggs from the wet treatment group was lightly blotted to remove surface water that was not tightly held on or within the egg chorion by capillary action. Groups of 3–5 eggs were attached to the tip of a 36-gauge copper-constantan thermocouple with a light coating of petroleum jelly, and positioned in two 10-ml disposable pipette tips, nested so that the eggs were located in the lower pipette tip. The tip of the thermocouple was threaded through the upper pipette tip and positioned next to the eggs to detect exotherms produced when the eggs froze. The temperature at which an exotherm was first detected was recorded as the supercooling point. Each unit was placed in a glass test tube (13 × 100 mm) supported by a foam platform, and a foam plug was used to seal the upper part of each tube. The foam platform was lowered into a Neslab RTE 140 refrigerated ethanol bath that was initially set at 0 °C, and cooled at 1.5 °C min^{-1} until all the eggs froze.

Post-exposure effect of moisture on hatchability

For both western and northern corn rootworms, three replications of 100 eggs for each moisture treatment were exposed for 1 h to temperature treatments of –12.0, –17.5, and –21.5 °C. Eclosed larvae and dead eggs were counted and removed daily under incubation at 25 °C.

Results

Moisture treatments

Eggs of western and northern corn rootworms responded differently to moisture treatment. Western corn rootworm eggs lost more moisture in the dry treatment than did northern corn rootworms (Table 1). The mean weight of

Table 1 Mean weight ± SE of northern and western corn rootworm eggs subjected to three moisture treatments

	Treatment			
Population	Wet (mg)	Moist (mg)	Dry (mg)	ANOVA
Northern	60.5 ± 2.5	57.0 ± 1.1	53.2 ± 3.0	$F_{2,14} = 2.5$, $P = 0.13$
Western	58.5 ± 1.5	61.3 ± 2.2	32.3 ± 3.1[a]	$F_{2,14} = 44.7$, $P<0.0001$

[a]Significantly different from other moisture treatments within the western corn rootworm population (Bonferroni P<0.001).

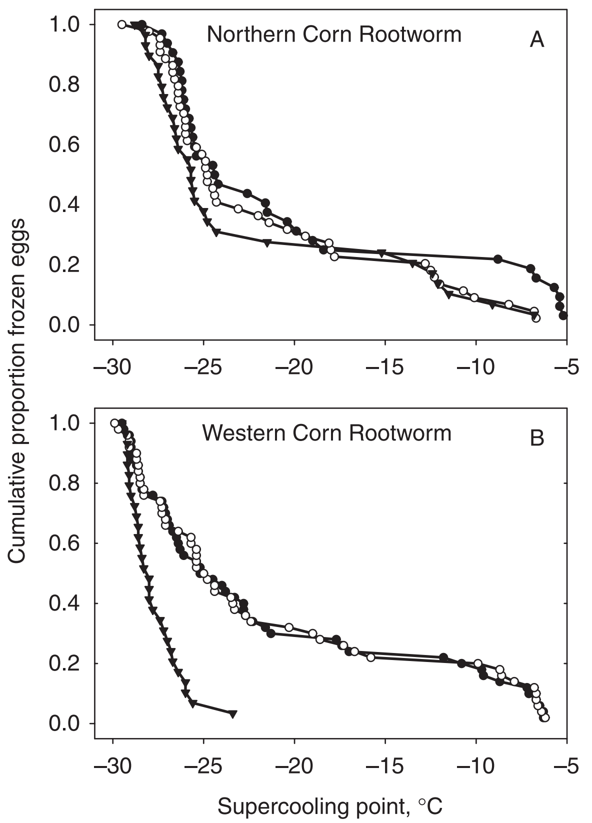

Figure 1 Cumulative proportion of northern (A) and western (B) corn rootworm eggs frozen during cooling following treatment at three moisture levels. Treatments were: ▼ dry (0 ml), ○ moist (1.5 ml), and ● wet (3.0 ml).

western corn rootworm eggs in the dry treatment was significantly lower ($F_{2,14} = 44.7$, $P<0.001$) than the mean weights in the wet or moist treatments. The weights of the northern corn rootworm eggs did not differ between the dry, moist, or wet treatments.

Supercooling determinations

Some eggs of both species of rootworm supercooled to as low as –27 °C. Moisture treatment prior to supercooling had little effect on northern corn rootworm eggs (Figure 1A). Mean supercooling points for northern corn rootworm eggs were –20.0 ± 1.4 °C (n = 32) for the wet treatment, –21.8 ± 3.3 °C (n = 44) for the moist treatment, and –22.8 ± 4.2 °C (n = 29) for the dry treatment. Western corn rootworm eggs were more resistant than northern corn rootworm eggs to the effects of desiccation followed by supercooling, i.e., after drying, western corn rootworm eggs lost more water and supercooled to a lower temperature than did northern corn rootworm eggs (Figure 1B). Mean supercooling points for western corn rootworm eggs were –21.8 ± 1.1 °C (n = 50) for the wet treatment, –21.7 ± 1.1 °C (n = 50) for the moist treatment, and –27.8 ± 0.3 °C (n = 29) for the dry treatment. Results of a Kolmogorov–Smirnov (KS) distribution test indicated that the distribution of supercooling points below about –22 °C for western corn rootworm eggs subjected to the dry treatment was significantly different from those of eggs subjected to wet conditions (KS = 2.17, P = 0.0002) or moist conditions (KS = 2.41, P = 0.0001). Above –22 °C, the cumulative distributions of supercooling points for western corn rootworm eggs did not differ significantly among the moisture treatments.

Egg hatchability

Northern corn rootworm eggs maintained 20–50% hatchability following 1 h exposure to –17.5 °C, but less than 10% hatchability at –21.5 °C (Figure 2A). Moisture treatment progressively lowered the hatchability of northern corn rootworm eggs following the –12.0 and –17.5 °C treatments. Western corn rootworm eggs were more

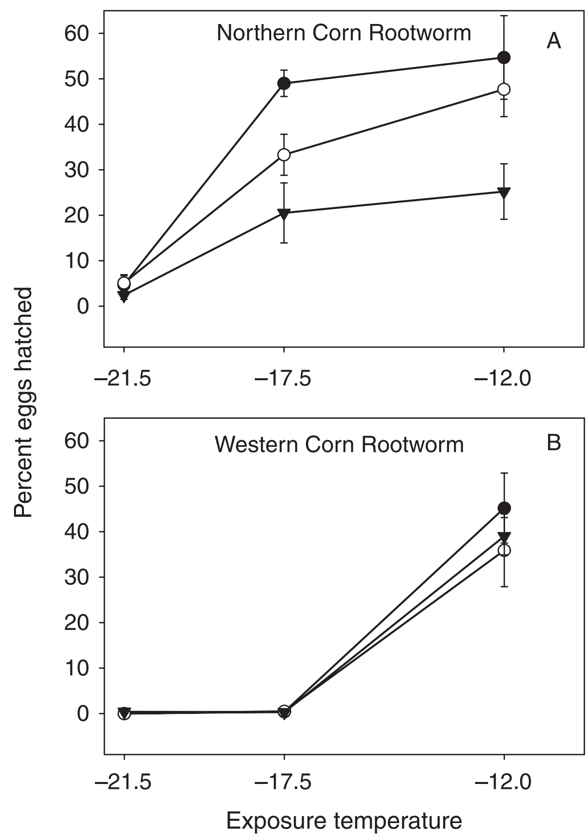

Figure 2 Per cent of northern (A) and western (B) corn rootworm eggs that hatched after exposure to subfreezing temperatures at three moisture levels. Treatments were: ● dry (0 ml), ○ moist (1.5 ml), and ▼ wet (3.0 ml).

sensitive to cold treatment, but moisture treatment had little effect on their hatchability (Figure 2B). About 40% of western corn rootworm eggs hatched in the −12.0 °C treatment, regardless of the moisture regime, and all eggs failed to hatch in the 17.5 and −21.5 °C treatments.

Discussion

Northern and western corn rootworm eggs responded quite differently to sub-zero temperatures following moisture treatment. Northern corn rootworms resisted freezing and chilling injury following desiccation better than western corn rootworms (Figures 1 and 2). In the dry treatment, western corn rootworm eggs lost more water than northern corn rootworms (Table 1), and supercooled to a greater extent (Figure 1B). Fewer eggs of northern than of western corn rootworms froze when cooled to above ca. −12.0 °C (Figure 1). These results suggest that northern corn rootworms are better adapted for survival in northern climates where the soil temperature routinely reaches −12.0 °C during the winter. These data are consistent with the fact that northern corn rootworms were indigenous to the tall-grass prairie regions of North America prior to the introduction of corn as an agricultural crop in the late 19th century. The ability of western corn rootworm eggs to supercool (survive chilling) under desiccation also is consistent with the premise that resistance to desiccation may pre-adapt organisms of tropical origin (e.g., western corn rootworm) for survival in colder regions (Ellsbury et al., 1998).

Although freezing is likely to be a lethal event for corn rootworm eggs of both species (Gustin, 1981) our results suggest that pre-freezing chill injury also causes significant mortality. About 80% of northern corn rootworm eggs endured supercooling under all three moisture treatments down to −12.0 °C and −17.5 °C, whereas 50–80% of northern corn rootworm eggs failed to hatch when subjected, even briefly, to temperatures of −12.0 or −17.5 °C. The higher moisture treatment increased the susceptibility of northern corn rootworm eggs to pre-freezing lethal chill injury (Figure 2A). Western corn rootworm eggs, with a hatching rate of about 40% after exposure to −12.0 °C and no hatch at −17.5 °C (Figure 2B), were more sensitive than northern corn rootworms to pre-freezing chill injury, regardless of moisture treatment. For both species, the lower lethal temperature limits for survival probably occur well above the supercooling point for most eggs. As Bale (1987) observed in a review of insect cold hardiness, from an ecological standpoint, the determination of supercooling capacity is necessary but not always sufficient for characterizing the limit of cold tolerance. The level of cold tolerance that we observed was consistent with the observations of Gustin (1981), that minimum temperatures below −10 °C and approaching −13 °C during a 5-week period in December to January caused a significant mortality to eggs of *D. virgifera*.

While we acknowledge that moisture treatments on a filter paper substrate only partially simulate naturally occurring soil conditions, our results nonetheless suggest that moisture and temperature interact to influence overwinter egg survival, particularly of northern corn rootworms. This concept contrasts with the presumption of Calkins & Kirk (1969), that desiccation from lack of winter precipitation during field studies was a primary mortality factor for overwintering eggs of both northern and western corn rootworms. The negative effect of moisture on the supercooling capacity of rootworm eggs supports the regression model of Turpin et al. (1972), who identified poorly drained sites and sites without steep slopes as predictors of locales that are less likely to incur damage by corn rootworms. The data presented herein are also consistent with our previous observations (Ellsbury et al., 2001) in a corn/soybean rotational system, showing that northern corn rootworm infestations were lowest at poorly drained sites. Differential winter egg mortality occurring at different landscape positions within a given production field may be a primary mechanism mediating spatial variability in corn rootworm populations. Moreover, the contrasting results for northern and western corn rootworms reported herein and in earlier literature (Woodson & Gustin, 1993; Woodson & Ellsbury, 1994) suggest that a site-specific estimation of infestation and damage potential based on winter egg survival for western and northern corn rootworms will require distinctly different predictive models for the two species.

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